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IgM and C3.

④ These data indicate that the intravascular mass of immunoglobins does not change during prolonged exercise in the heat, and that hypohydration results in a translocation of C3 to the intravascular space.

↓ Key word: Dehydration. (RW) X

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**HUMAN INTRAVASCULAR IMMUNOGLOBIN RESPONSES TO EXERCISE-HEAT  
AND HYPOHYDRATION**

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## **Summary**

1. The effects of prolonged treadmill exercise in the heat and hypohydration on changes in the intravascular mass of immunoglobins were examined.

2. Five heat acclimated males attempted two Heat Stress Tests (HSTs). One HST was completed when subjects were euhydrated and the other HST when subjects were hypohydrated (-5% from base line body weight). The HSTs consisted of 30 min of rest in a 20°C antechamber, followed by a 120-min exposure (2 repeats of 15 min rest and 45 min walking) in a hot (35°C, 45% rh) environment.

3. The following observations were made concerning immunoglobulin responses to hypohydration and exercise-heat stress: A) the changes in concentration ( $\text{mg} \cdot \text{dl}^{-1}$ ) of the measured immunoglobins were usually a direct reflection of changes in the plasma volume; B) hypohydration increased the intravascular mass (g) of the complement enzyme C3, but did not alter the intravascular mass of IgG, IgA and IgM; and C) prolonged treadmill exercise in the heat, when either euhydrated or hypohydrated, did not alter the intravascular mass of IgG, IgA, IgM and C3.

4. These data indicate that the intravascular mass of immunoglobins does not change during prolonged exercise in the heat, and that hypohydration results in a translocation of C3 to the intravascular space.

**Key Words:** Dehydration, Complement Enzyme, C3, IgG, IgA, IgM, Immunological Responses, Plasma Volume

**Abbreviations:** HST, Heat Stress Test

## Introduction

Immunoglobins (or antibodies) are proteins synthesized by plasma cells and distributed throughout the extracellular space (1,2). Since these proteins contribute little to the plasma oncotic pressure and are difficult to measure, few investigators have examined intravascular immunoglobulin changes during prolonged exercise-heat stress (3-8). Although possible, it is doubtful that changes in the immunoglobulin intravascular mass would represent the synthesis from an antigen challenge during acute exercise-heat stress. During exercise in the heat, changes in the intravascular masses of various immunoglobins would probably reflect exchanges with extravascular pools as well as the perfusion of different intravascular beds. Exercise will increase skeletal muscle perfusion, and heat stress will increase cutaneous blood flow and volume; whereas splanchnic blood flow will be decreased by exercise, heat stress and hypohydration (9-11). Immunoglobulin fractions have relatively large molecular weights and are distributed to different tissues and intravascular beds. For example, IgG ( $\sim 150 \times 10^3$  daltons) is distributed throughout the extracellular spaces, IgA ( $\sim 170 \times 10^3$  daltons) is distributed throughout the gastrointestinal tract, IgM ( $\sim 900 \times 10^3$  daltons) is distributed primarily within the intravascular space and the complement enzyme C3 ( $\sim 185 \times 10^3$  daltons) is distributed throughout the body (1). Therefore, as a result of different distribution and perhaps extravascular pool sizes, and reflection coefficients, the different immunoglobulin fractions may have varied response patterns to hypohydration and during exercise in the heat.

Several investigators have examined plasma concentrations of various immunoglobins both before and immediately after running a race (7,8) or prolonged cycle exercise (3,5,6). Poortmans and Haralambie (7) found an increased plasma concentration of IgG with no change in IgA despite an estimated increase in plasma volume after a 100 km race. As a result, the intravascular mass of IgG, and perhaps IgA, was increased after the race. Rocker and associates (8) reported no change in

the intravascular mass of IgG and IgM after a 32 km race in an unspecified environment. During the race, their subjects had dehydrated by 4% of their body weight. Therefore, the lack of a change in the intravascular mass of these immunoglobins (8) possibly represents the dual actions of hypohydration and the submaximal running. De Lanne and associates (3) reported that immunoglobins are probably lost from the intravascular space during 30 min of cycle exercise in comfortable and hot environments. In two studies, Haralambie and colleagues (5,6) found that the plasma concentration of the immunoglobulin fractions IgG, IgA and IgM did not change despite an estimated plasma volume reduction during either 1 h (5) or 2 h (6) of submaximal cycle exercise in unspecified environmental conditions. As a result, both of these studies' data (5,6) suggest a reduced intravascular mass of immunoglobins during prolonged submaximal cycle exercise.

The immunoglobulin data from these previous studies are difficult to interpret because of several methodological concerns. Evidence of adequate controls (9,10) for body posture and arm position when obtaining the pre- and post-exercise blood samples have not been provided (3,4-8). For example, differences in posture between samples will alter the protein concentration of the blood sample (9,10). Also, some studies did not correct for plasma volume changes (4,5), or used indices that are probably not valid to estimate changes in plasma volume (3,6); and only the study of Rocker *et al.* (8) actually measured plasma volume so that the intravascular mass of immunoglobins could be calculated. Therefore, these studies cannot accurately discern if the change in immunoglobulin concentration is due to changes in plasma volume or the immunoglobulin intravascular mass.

In the present paper, we examined the intravascular masses of IgG, IgA, IgM and C3 during rest and exercise in the heat. Experiments were conducted when subjects were both euhydrated and hypohydrated. It was anticipated that the additional perturbation of hypohydration would modify the immunoglobulin redistribution

by altering perfusion of the cutaneous and splanchnic vascular beds. Hypohydration has been reported to modify the total circulating protein mass redistribution to exercise-heat stress (9,10). Knowledge obtained from this paper will help to clarify the immunological redistribution to exercise-heat exposure, and to provide insight on the normal distribution of these proteins.

## Methods

**Subjects.** Five fit male volunteers participated in this investigation. They gave their voluntary and informed consent to participate in this investigation, which had received approval by the appropriate Institutional Review Boards. The subjects had a mean ( $\pm$ SD) age of  $33 \pm 2$  yr, weight of  $86 \pm 13$  kg, surface area-to-mass ratio of  $234 \pm 14$   $\text{cm}^2 \cdot \text{kg}^{-1}$ , percent body fat of  $20 \pm 5$  and maximal aerobic power of  $52 \pm 8$   $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ .

**Protocol.** Initially, the subjects were familiarized with the test procedures, had their percent body fat determined by hydrostatic weighing and completed a maximal aerobic power test (12). In addition, on fifteen days prior to experimental testing and throughout the study, nude body weights were measured in the morning after voiding and before breakfast. These body weights were used to establish base line body weights that represented euhydration for each subject.

Subjects were heat acclimated by performing treadmill exercise (0% grade at  $1.34 \text{ m} \cdot \text{s}^{-1}$ ) for 120 minutes on nine days in a hot-dry ( $45^\circ\text{C}$  ambient temperature, 20% relative humidity,  $33^\circ\text{C}$  wet bulb globe temperature) environment. During all tests, the subjects wore gym shorts, and tennis shoes; the subjects drank water ad libitum during the acclimation sessions. Following the nine-day heat acclimation program, the subjects had their resting plasma volume measured on one day (while euhydrated) and then on other days completed two HSTs. One HST test was done while euhydrated, and the other HST test was done while hypohydrated by 5% of

body weight. The hypohydration HST was conducted 48 h after the euhydration HST. The HSTs were conducted in a hot ( $35^{\circ}\text{C}$  ambient temperature, 70% relative humidity,  $28^{\circ}\text{C}$  wet bulb globe temperature) environment. This environment was selected to potentiate evaporative and provide limited convective and radiative heat exchange. Each HST was 120 min (2 repeats of 15 min rest and 45 min exercise) in duration. During exercise, subjects walked ( $6\%$  grade,  $1.34\text{ m}\cdot\text{s}^{-1}$ ) on a treadmill, and during the rest periods they were weighed and rehydrated with slightly chilled ( $\sim 20^{\circ}\text{C}$ ) spring water to maintain their desired body weight (i.e., euhydration or hypohydration level).

Approximately 24–48 h prior to each hypohydration HST, subjects initiated a program of voluntary food and fluid restriction. Also, in the afternoon the day before the hypohydration HSTs, subjects performed light-intensity exercise in a hot environment to dehydrate to their target body weight (5% below base line). In addition, as a control the subjects performed exercise in the heat, but with adequate rehydration, on the day prior to the euhydration HSTs. After achieving the target body weight, subjects were removed to a comfortable environment to spend the night. During the night, subjects were allowed fresh fruit and juice, but only in amounts that allowed maintenance of their desired body weight. All subjects completed the dehydration 15–18 h prior to the HSTs. This 15–18 h period was spent to provide time for fluid compartments to achieve equilibration at the achieved hydration level. The following morning, the subjects were weighted (0700 h), provided fresh fruit and juice (as weight allowed), instrumented and tested (0930 h). These dehydration procedures are consistent with those of previous investigations (12–15).

**Measurements.** Electrocardiogram was obtained with chest electrodes (CM5 placement) and radiotelemetered to an oscilloscope-cardiotachometer unit (Hewlett-Packard). During the maximal aerobic power tests, an automated system (Sensormedics Horizon (MMC)) was used to measure oxygen uptake. During the



HSTs, the respiratory gases were collected in 150-liter Douglas bags. The volume of expired gases was measured with a Tissot gasometer, and the oxygen and carbon dioxide concentrations were measured with an electrochemical oxygen analyzer (Applied Electrochemistry S-3A) and an infrared carbon dioxide analyzer (Beckman LB-2), respectively. During the HSTs, rectal temperature was measured from a thermistor inserted ~10 cm beyond the anal sphincter.

Venous blood samples were collected from an indwelling Teflon catheter placed within a superficial forearm vein. Patency was maintained with heparinized saline; the catheter (2 ml of dead space) was flushed with 3 ml of blood before each 8 ml sample was obtained. Blood samples taken at rest were obtained while all the subjects stood (for 20 min prior to sampling) in the antechamber (20°C ambient temperature, 40% relative humidity), and exercise blood samples were obtained 15 min and 40 min into each exercise bout while the subjects continued to walk. All blood samples were obtained with the catheterized arm hanging in a relaxed manner. Triplicate measurements were made for all blood variables. Automated systems were used to measure hemoglobin (Hemoglobinometer, Coulter Electronics), and plasma osmolality was measured by a vapor pressure osmometer (Model 5500, Wescor). The plasma immunoglobins (Ig) were measured on a nephelometer (Beckman, Automated Immunochemistry System) employing a commercially available immunoglobulin reagent kit (Beckman, Cat. #662901). Plasma volume at rest (euhydrated) was measured by the iodine-labeled ( $^{125}\text{I}$ ) albumin method (17). The percent change in plasma volume was calculated from the appropriate hemoglobin and hematocrit values (18). The plasma volumes during exercise were calculated by adjusting the measured plasma volume at rest (while euhydrated) by the appropriate percent change in plasma volume. The immunoglobulin intravascular mass was calculated as the product of plasma volume and the immunoglobulin concentration ( $\text{mg} \cdot \text{dl}^{-1}$ ).

**Statistical Analyses.** Mean  $\pm$ SD, simple regression and analyses of variance (ANOVA) for repeated measures (subjects  $\times$  time  $\times$  treatment) were used. When significant main effects or interactions were found, the critical differences were calculated by Tukey's procedure. For one subject, missing data points were calculated for the last blood sample during the hypohydration experiments (19).

## Results

The final exercise rectal temperature and heart rate values did not change between the final five of the nine-day heat acclimation days, indicating complete heat acclimation. During the euhydration experiment, the subjects had a mean  $\pm$ SD body weight of  $87.2 \pm 12.9$  kg. During the hypohydration experiments, the subjects' body weights were  $82.8 \pm 12.2$  kg, which corresponded to  $5.0 \pm 0.0$  percent below their base line body weight. Plasma osmolality values at rest were greater ( $P < 0.01$ ) when hypohydrated ( $293 \pm 6$  mosmol $\cdot$ kg $^{-1}$ ) than when euhydrated ( $283 \pm 1$  mosmol $\cdot$ kg $^{-1}$ ).

During the Heat Stress Tests, the subjects had a mean  $\pm$ SD metabolic rate of  $656 \pm 83$  watts, which corresponded to  $42 \pm 6\%$  of their maximal aerobic power. Metabolic rate values were not different between the euhydration and hypohydration HSTs. The final exercise rectal temperatures were greater ( $P < 0.05$ ) during the hypohydration ( $38.5 \pm 0.5^{\circ}\text{C}$ ) than euhydration ( $37.8 \pm 0.3^{\circ}\text{C}$ ) HSTs; likewise, total body sweating rate values were lower ( $P < 0.05$ ) during the hypohydration ( $903 \pm 97$  g $\cdot$ min $^{-1}$ ) than euhydration ( $1,029 \pm 67$  g $\cdot$ min $^{-1}$ ) HSTs. The final exercise heart rate values were greater ( $P < 0.05$ ) during the hypohydration ( $150 \pm 17$  bpm) than euhydration ( $130 \pm 18$  bpm) HSTs.

Figure 1 presents the subjects' plasma volume responses during rest and the two HSTs. Although resting values were similar, plasma volumes were lower ( $P < 0.01$ ) when hypohydrated than when euhydrated during each exercise bout. Figure 2 presents the subjects' immunoglobulin concentrations during the two HSTs. The IgG

and IgA concentrations were greater ( $P < 0.05$ ) when hypohydrated than when euhydrated. The IgM concentration was not altered ( $P > 0.05$ ) by hydration or exercise. Finally, the C3 concentration was greater ( $P < 0.05$ ) when hypohydrated than when euhydrated. Table 1 presents the immunoglobins' intravascular mass during rest and the two HSTs. The intravascular mass of IgG, IgA and IgM were not altered ( $P > 0.05$ ) by hydration or exercise. The C3 intravascular mass was greater ( $P < 0.05$ ) when hypohydrated than when euhydrated. This significant increase when hypohydrated was primarily due to the elevated resting and not exercise values.

### Discussion

The experimental design allowed an evaluation of the redistribution of intravascular immunoglobins during prolonged exercise, which was performed at several different levels of plasma volume and degrees of heat strain. This hypohydration program was successful in causing substantial differences (~12% by the end of exercise) in plasma volume between the two HSTs. In addition, hypohydration was used to elevate body temperature (~0.7°C by the end of exercise) and increase thermal strain (10).

We found that the intravascular mass of IgG, IgA, IgM and C3 was not altered by prolonged treadmill exercise-heat stress or hypohydration. Previous studies have suggested that the intravascular mass of immunoglobins may increase after prolonged race running (3,7,8) and decrease for prolonged cycle exercise (5,6). Those studies, however, did not provide evidence of adequate controls for blood sampling procedures (3,5-8) and often used invalid indices to estimate changes in plasma volume (3,6). As a result, their results are difficult to interpret. In fact, these concerns are emphasized by our findings that changes in the plasma concentration of immunoglobulin proteins are often the reflection of plasma volume changes. On the other hand, the present study's results may not be directly applicable to race running or cycle

exercise. Race running would be expected to elicit a fairly high (70-90%  $\dot{V}O_2$  max) metabolic intensity, whereas the present study employed a moderate (42%  $\dot{V}O_2$  max) exercise intensity. During high intensity running, the immunoglobulin intravascular mass may increase due to greater fluid exchange with the interstitial space resulting in greater lymph turnover than during moderate intensity treadmill exercise. Finally, prolonged cycle exercise, unlike treadmill, has a secondary hemoconcentration that is associated with the loss of plasma protein to the interstitial space (9,10). As a result, it might be that the intravascular immunoglobulin mass would also decrease over time during prolonged cycle exercise, unlike treadmill walking or race running.

The present investigation is the first to determine the effects of either prolonged exercise, heat stress or hypohydration on changes in the C3 intravascular mass. Of the measured immunoglobulins, C3 along with IgG have the lowest reflection coefficients, and therefore would be the most likely to show changes in intravascular mass during the studied perturbations. The C3 intravascular mass was greater ( $P < 0.05$ ) during rest and exercise when hypohydrated than when euhydrated. The physiological implications of, and mechanism responsible for, the slightly greater C3 intravascular mass is unclear. A 5% level of hypohydration should result in a substantial (30-40%) reduction of the interstitial fluid volume (10). The movement of interstitial fluid into the intravascular space may have resulted in the translocation of C3. It is doubtful that the increased C3 intravascular mass represents a response to inflammation or to lyse target cells.

Several investigators have examined the effects of passive thermal dehydration on plasma immunoglobulin levels (20-22). Ohira and colleagues (21) dehydrated six subjects by 1.2% of their body weight during 30 min in a 75°C sauna bath. They reported that thermal dehydration caused an 11% increase of immunoglobulins even after correcting for the observed plasma volume reduction. Those investigators suggested that immunoglobulin mobilization from the interstitial spaces may represent a

"nonspecific" stress response (21). On the other hand, two investigators reported that passive thermal dehydration did not alter the intravascular immunoglobulin mass (20,22). Myhre and Robinson (20) exposed two groups of six subjects to a 50°C sauna both for 4 hours. One group did not ingest fluids during the exposure and dehydrated by ~3% of their body weight while the other group maintained euhydration. Stephenson and colleagues (22) exposed five subjects for 30 min to an 80°C sauna bath on two occasions. During one occasion, they dehydrated by ~1% of their body weight, while on the other occasion they maintained euhydration. Each of these studies concerning the effects of passive thermal dehydration on plasma immunoglobulin levels employed subjects who were unacclimated to the heat (20-22).

The present investigation used a fairly severe level of hypohydration (5% reduction in body weight) which was achieved by the combination of exercise with heat exposure. In addition, the present subjects were heat acclimated and served as their own controls for the euhydration and hypohydration experiments. We found no evidence to support the idea that changes in IgG, IgA and IgM may provide a stress index for the perturbation of dehydration.

We have made several observations concerning immunoglobulin responses to hypohydration exercise-heat stress: A) that changes in the concentration of the measured immunoglobins were usually a direct reflection of changes in the plasma volume; B) hypohydration increased the intravascular mass of the complement enzyme C3, but did not alter the intravascular mass of IgG, IgA and IgM; and C) prolonged treadmill exercise in the heat, when either euhydrated or hypohydrated, will not alter the intravascular mass of IgG, IgA, IgM and C3. These data indicate that the intravascular mass of immunoglobins does not change during prolonged exercise in the heat, and that hypohydration results in the translocation of C3 to the intravascular space.

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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.

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Table 1. Intravascular Mass (g) of Immunoglobins at Rest and Exercise During the Euhydration and Hypohydration Heat Stress Tests.

Variable	EUYDRATION						HYPOHYDRATION					
	Rest	Ex Bout 1		Ex Bout 2		Rest	15 min	Ex Bout 1		15 min	Ex Bout 2	
		15 min	40 min	15 min	40 min			40 min	15 min		15 min	40 min
IgG $\bar{x}$	34	35	36	35	35	36	34	33	33	33	33	35
SD	5	8	9	10	9	12	9	10	11	9		
IgA $\bar{x}$	6.5	6.7	6.6	6.5	6.5	6.9	6.4	6.5	6.7	6.7	6.7	6.7
SD	3.4	3.0	3.1	3.1	3.0	3.2	2.7	3.3	3.3	3.3	3.2	3.2
IgM $\bar{x}$	4.5	4.7	4.6	4.5	4.4	4.6	4.4	4.3	4.3	4.3	4.3	4.3
SD	1.0	1.2	1.3	1.1	0.9	1.2	1.1	1.0	1.0	1.0	1.0	1.0
C3 $\bar{x}$	3.9	4.5	4.5	4.2	4.2	4.8	4.5	4.5	4.6	4.6	4.6	4.6
SD	0.5	1.3	1.2	1.1	0.9	1.0	1.3	1.2	1.1	1.1	1.0	1.0

min is minutes; EX is exercise.

### Figure Legends

**FIGURE 1** Plasma volume responses ( $\bar{X} \pm SE$ ) at rest and exercise during the two experimental conditions.

**FIGURE 2** The plasma concentration of the immunoglobulin IgG, IgA, IgM and C3 at rest and exercise during the two experimental conditions. SE bar, mean value for all observation times in each treatment.



